Allopurinol and Nitric Oxide Activity in the Cerebral Circulation of Those With Diabetes

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A randomized trial

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OBJECTIVE — Type 2 diabetes increases risk of stroke, perhaps because of impaired cerebrovascular basal nitric oxide (NO) activity. We investigated whether this activity is improved by a 2-week course of the xanthine oxidase inhibitor allopurinol.

RESEARCH DESIGN AND METHODS — We performed a randomized, double-blind, placebo-controlled crossover study. We measured the response to infusion of NG-monomethyl-L-arginine (L-NMMA) in males with type 2 diabetes before and after allopurinol or placebo. The primary end point was the change in internal carotid artery flow following L-NMMA infusion, expressed as the area under the flow-per-time curve.

RESULTS — We enrolled 14 participants. Allopurinol improved responses to L-NMMA when compared with responses associated with placebo ($P = 0.032$; median reduction in internal carotid artery flow following L-NMMA of 3,144 ml [95% CI 375–7,143]).

CONCLUSIONS — Xanthine oxidase inhibition with allopurinol appears to improve cerebral NO bioavailability, as evidenced by a greater response to infusion of L-NMMA.

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Type 2 diabetes conveys an increased risk of stroke (1,2), perhaps because of impaired cerebrovascular basal nitric oxide (NO) bioavailability—a hypothesis suggested by impaired response to infusion of the endothelial NO synthase inhibitor NG-monomethyl-L-arginine (L-NMMA) (3). We performed a randomized, double-blind, placebo-controlled crossover study to test whether this response is improved following a course of the xanthine oxidase inhibitor allopurinol.

RESEARCH DESIGN AND METHODS

— The study was approved by the West Medical research ethics. Male patients aged >40 years with type 2 diabetes of duration <5 years and A1C <9.0% were studied. Those with severe extracranial internal carotid artery (ICA) stenosis, known as coronary arterial disease, and those receiving insulin were excluded. All gave written informed consent and underwent exercise tolerance testing (ETT) to exclude subclinical coronary arterial disease.

Baseline assessment of ICA flow and middle cerebral artery mean flow velocity was performed. Peripheral (radial) pulse-wave analysis (PWA) and (carotid-radial) pulse-wave velocity (PWV) were measured. Blood was drawn for measurement of routine parameters and vascular endothelial growth factor, soluble intercellular adhesion molecules, E-selectin, and C-reactive protein levels. Thereafter, a 45-ml infusion of 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ clinical-grade L-NMMA (Clinalfa/Bachem-Ag, Weil-am-Rhein, Germany) dissolved in normal saline was given intravenously over 15 min. ICA, middle cerebral artery, PWA, and PWV parameters were repeated upon cessation of infusion and 10, 20, and 30 min thereafter.

Participants were then randomized to either 300 mg allopurinol or a matching placebo, each taken orally once daily for 2 weeks. Following this, all assessments were repeated. A 2-week washout period then ensued, after which repeat assessment, a 2-week dose of the other agent (placebo or allopurinol), and the final assessment occurred.

The primary end point was change in ICA flow induced by the L-NMMA infusion and expressed as area under the flow-per-time curve (AUC) measured from the start of infusion to 20 min after its completion (3,4). A negative AUC signifies the expected reduction in ICA flow following L-NMMA infusion. Based on our previous pilot data (3), a sample of 20 patients would enable detection of a clinically significant improvement in L-NMMA responsiveness following allopurinol (to ~75% of that seen in nondiabetic individuals) with 90% power ($\acute{a} = 5\%$). Secondary end points were change in augmentation index (measured during PWA), PWV, and blood markers.

Standard crossover study analysis techniques were employed, and differences between the study periods were compared using paired nonparametric tests (Wilcoxon's signed-rank test). A positive difference between study periods represents improvement in favor of allopurinol. A mixed-effects model was generated to adjust for any effect of treatment ordering.

RESULTS

— Fourteen patients were recruited. Ten completed the protocol; one had a positive ETT, two failed to at-

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Table 1—*Change in hemodynamic parameters: response to L-NMMA during study periods*

Variable	Baseline visit	Change postplacebo	Change postallopurinol	Difference between periods*	*P*
ICA flow (ml)†	−789 (−4,194 to 735)	−1,314 (−3,359 to 419)	1,134 (−964 to 7,872)	3,144 (375 to 7,143)	0.032
Systolic blood pressure (mmHg)†	−133 (−205 to 94)	20 (−260 to 158)	116 (−248 to 501)	14 (−461 to 588)	0.919
Diastolic blood pressure (mmHg)†	66 (−60 to 276)	−55 (−220 to 86)	−162 (−206 to 94)	−56 (−218 to 90)	0.476
Middle cerebral artery flow velocity (ml/s)†	14 (−29 to 53)	−41 (−112 to 108)	−38 (−146 to −9)	−35 (−174 to 45)	0.359
Augmentation index (%)‡	23.5 (18.5–29.5)	−3 (−14.5 to 4.5)	2 (−6.00 to 10)	−4.3 (−11 to 7)	0.56
PWV (m/s)‡	9 (7.6–9.6)	0.8 (0.1–10.2)	0.75 (−0.85 to 2.3)	−0.32 (−2.1 to 7.8)	0.39
Vascular endothelial growth factor (pg/ml)‡	90.4 (42.9–226.3)	−14 (−67 to 59.1)	66.9 (5.9–119.6)	91 (−53 to 151)	0.083
Soluble intercellular adhesion molecules (ng/ml)‡	414 (336–555.8)	73.6 (−85.3 to 96.5)	9 (−7.8 to 65.3)	−51.7 (−81 to 91.9)	0.689
E-selectin (ng/ml)‡	85.1 (63.8–116.3)	0.01 (−14.6 to 10.3)	2.4 (−12.9 to 17.1)	16 (−19 to 26)	0.45
C-reactive proteins (mg/l)‡	1.02 (0.5–1.1)	0.09 (−0.3 to 0.75)	0.195 (−0.01 to 1)	0.33 (−0.48 to 1.29)	0.398

Data are median (interquartile range) unless otherwise indicated. *Data are estimated differences in medians (95% CI) by Wilcoxon's signed-rank test. †Data refer to the change in area under the time curve in response to L-NMMA. ‡Data refer to the difference between preinfusion values. *P* values were determined by Wilcoxon's signed-rank test.

tend after the ETT and the first visit, and one was unable to receive L-NMMA during his last study visit. The study was terminated early as a result of the national lack of availability of L-NMMA. No adverse events occurred.

Of those recruited, mean ± SD age was 53.1 ± 10.8 years, 85.7% (n = 12) had coexisting hypertension, 78.6% (n = 11) were receiving oral hypoglycemic agents, 78.6% were receiving ACE inhibitor or angiotensin receptor blocker therapy, 64.3% were receiving antiplatelet therapy, and 92.3% were on lipid-lowering therapy. Mean A1C was 6.6 ± 0.94%.

The baseline response to L-NMMA infusion was impaired. ICA flow fell by a median of 4.8% (95% CI −17.3 to 9.4; P = 0.25). We saw no change in blood pressure parameters where AUCs were considered or in the augmentation index or PWV following L-NMMA (Table 1).

Allopurinol treatment significantly augmented the reduction in ICA flow following L-NMMA infusion (Table 1). ICA flow fell by a median of 11.9% (95% CI −2.5 to 23.3; P = 0.04) following L-NMMA after treatment with allopurinol. The mixed-effects model revealed results consistent with the main analysis (P = 0.046 for allopurinol vs. placebo) and found no significant order effect (P = 0.51). Allopurinol led to an improvement in response to L-NMMA in 8 of the 10 individuals.

The preinfusion augmentation index, PWV (Table 1), and L-NMMA (data not shown) did not differ significantly between the treatment periods (Table 1). No significant differences were seen in any of the blood markers (Table 1).

CONCLUSIONS — L-NMMA reduces cerebral blood flow through restriction of NO activity. The higher the basal NO activity, the larger the effect. Treatment with allopurinol enhanced this effect, implying that allopurinol improves basal levels of NO activity, causing them to approach those previously seen in healthy volunteers (3).

We saw no improvement in blood markers or measures of peripheral arterial stiffness. These results contrast with a previous study where forearm blood flow responses were improved following allopurinol (5), but we employed different techniques and did not design our study to detect differences in these parameters. Interestingly, at the baseline visit, we failed to replicate the increase in the augmentation index or PWV following L-NMMA seen previously in healthy volunteers (6), and it may be that this response is also impaired in those with diabetes.

The potential beneficial effects of allopurinol on the vasculature are twofold: it reduces xanthine oxidase–mediated O_2^- production (7) and reduces serum uric acid (8). Previous studies have shown a beneficial effect of xanthine oxidase inhibition on measures of forearm or coronary endothelial function (9), but this is the first human study to show improvement in cerebrovascular function.

We followed a rigorous protocol, and improvements in peripheral vascular responses to L-NMMA have been shown following treatment with other cardiovasculature-protective agents (10,11). However, we studied fewer patients than intended because of the lack of ongoing availability of L-NMMA, which reduces our statistical power, but the consistency of effect we saw is reassuring. Our dosing period was short, and we cannot exclude the possibility that the changes we saw may subsequently be reversed by other sources of O_2^-. Also, we have only examined the effect on endothelial NO synthase activity, and other forms of NO and O_2^- production may be important.

In summary, our data show that xanthine oxidase inhibition with allopurinol improves cerebral NO activity, which may benefit cerebrovascular health. We encourage further investigation of its use for stroke prevention.

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No potential conflicts of interest relevant to this article were reported.

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